FIELD ESTABLISHMENT OF FOURWING SALTBUSH
IN PROCESSED OIL SHALE AND DISTURBED NATIVE SOIL
AS INFLUENCED BY VESICULAR-ARBUSCULAR MYCORRHIZAE[1]

C. A. Call[2] and C. M. McKell[3]

ABSTRACT.— Seedlings of fourwing saltbush (*Atriplex canescens* (Pursh) Nutt.) were inoculated with indigenous vesicular-arbuscular mycorrhizal (VAM) fungi in a containerized system and transplanted into processed oil shale and disturbed native soil in a semiarid rangeland environment in northwestern Colorado. After two growing seasons in the field, plants inoculated with VAM had greater aboveground biomass, cover, and height than noninoculated plants. Mycorrhizal plants were more effective in the uptake of water and phosphorus. Infection levels of inoculated plants were greatly reduced in processed shale (from 13.0 at outplanting to 3.8 at harvest), but functional VAM associations could be found after two growing seasons. Results indicate that VAM help make processed oil shale a more tractable medium for the establishment of plants representative of later successional stages by allowing these plants to make effective use of the natural resources that are limiting under conditions of high stress.

Processed oil shale is biologically sterile, highly alkaline (pH of 8 to 12), highly saline (EC_e of 6 to 26 mmhos/cm), and deficient in available nitrogen (N) and phosphorus (P) (Institute for Land Rehabilitation 1979, Schmehl and McCaslin 1973). Relatively few studies have been conducted in the United States using untreated processed shale as a plant growth medium, and the results have generally been disappointing. Schmehl and McCaslin (1973) observed that germination and seedling growth of tall wheatgrass (*Agropyron elongatum* (Host) Beauv.) and Russian wildrye (*Elymus junceus* Fisch.) were significantly lower on processed shale than on soil. Merkel (1973) reported poor establishment of 10 direct-seeded grass, forb, and shrub species and several transplanted shrub species on untreated spent shale. In a similar study (Baker and Duffield 1973), several weedy species invaded untreated spent shale plots originally seeded with several shrub and perennial grass species.

Intensive treatments (leaching salts from the root zone, fertilization, mulching, and periodic supplemental irrigation) allow a variety of plant species to be directly established on processed shale (Bloch and Kilburn 1973, Harbert and Berg 1974). However, these treatments may not be feasible on a commercial scale due to their high cost or the unavailability of resources.

McKell (1978) proposed a strategy for the rehabilitation of processed oil shale piles that would work within the environmental constraints of affected arid and semiarid ecosystems and be more cost effective than those strategies that recommend extensive modifications of the spent shale. Local ecotypes of dominant plant species would be propagated, grown in containers, and transplanted into soil trenches surrounded by surface-treated water catchment slopes. Minimal fertilization and supplemental irrigation would be used only when necessary during the initial establishment period. Indigenous vesicular-arbuscular mycorrhizae may prove to be vital to the success of this minimum-treatment rehabilitation strategy.

Vesicular-arbuscular mycorrhizae (VAM) enhance plant absorption of P and other elements, enhance water uptake and transport in plants, and allow plants to withstand high temperatures (Gerdemann 1975). Vesicular-arbuscular mycorrhizae are also key links in nutrient cycling and energy flow processes in ecosystems (Trappe and Fogel 1977).

When soils are disturbed there is a reduction in VAM fungal propagules and a lower potential for infection of new host plants

(Miller 1979, Reeves et al. 1979). The greater the disturbance, the greater is the potential for elimination of mycorrhizal propagules and, therefore, a longer time is required for reestablishment of mycorrhizal vegetation.

The success of a minimal-treatment revegetation program for processed oil shale piles and disturbed soils may depend, in part, on developing methods to introduce VAM fungi into disturbed sites where they are absent. The inoculation of nursery bed or container media with VAM fungi, in combination with reduced fertility levels, could produce plants better adapted to the environmental conditions of the outplanting site and provide a source of inoculum for other desirable plant species that require VAM associations for successful establishment.

A field study was undertaken to determine if the inoculation of fourwing saltbush (*Atriplex canescens* (Pursh) Nutt.) with VAM fungi would provide plants better adapted for a minimal-treatment revegetation program for processed oil shale and disturbed native soil.

STUDY AREA

Upon initiation of this research in 1977, all field experiments were to be conducted on or adjacent to Federal Oil Shale Lease Tracts Ua and Ub in northeastern Utah. Due to the size of the study plot required, and the logistics and cost of hauling Paraho processed shale from the Anvil Points retort in northwestern Colorado to the Ua/Ub study site, field outplanting studies were moved to Anvil Points in 1979. Indigenous VAM fungal inoculum was collected on tracts Ua and Ub prior to the relocation of field outplanting studies to the Anvil Points site.

Tracts Ua and Ub are approximately 80 km southeast of Vernal, Utah, and are between 1520 and 1850 m above sea level. The climate of the area is semiarid, with annual precipitation ranging from approximately 230 to 280 mm. Shallow soils (less than 50 cm deep) on sloping to steep upland terraces are light-colored, moderately calcareous channery loams and channery sandy loams (VTN Colorado, Inc. 1977). Deep soils (greater than 150 cm deep) in drainage areas range from light-colored, moderately sandy or channery loams to strongly alkaline, highly saline,

sandy loams (VTN Colorado, Inc. 1977). Sagebrush-greasewood (*Artemisia tridentata* Nutt.-*Sarcobatus vermiculatus* (Hook.) Torr.), shadscale (*Atriplex confertifolia* (Torr. and Frem) Wats.), juniper (*Juniperus osteosperma* (Torr.) Little), and riparian vegetation types are found on tracts Ua and Ub (VTN Colorado, Inc. 1977). *Atriplex canescens* is found in all four vegetation types.

The Anvil Points Oil Shale Research Facility is 11.2 km west of Rifle, Colorado, and approximately 130 km southeast of tracts Ua and Ub in Utah. In 1975, a 55 x 122 m open area was filled with Paraho processed shale (maximum particle size of 3.8 to 6.5 cm) to a depth of 2.7 m (U.S. Dept. of Interior 1976). In 1978 two 50 cm-deep, v-shaped parallel trenches were constructed across the width of the shale pile and filled with a disturbed channery loam (Soil Conservation Service 1980), obtained adjacent to the site. The study site is 1722 m above sea level. The climate of the area is semiarid, with annual precipitation averaging 305 to 357 mm (Harbert and Berg 1974). The surrounding vegetation is the low elevation pinyon-juniper (*Pinus edulis* Engelm.–*Juniperus osteosperma* (Torr.) Little) woodland type (Ward et al. 1974).

METHODS

Isolation and Culture of Inoculum

During September 1977 5-kg soil samples were collected beneath three individuals of *A. canescens* on tracts Ua and Ub. Soil samples were maintained at 4 C until the time of processing a few days later. Spores of VAM fungi were collected from the soil using a wet-sieving and decanting technique (Gerdemann and Nicolson 1963), and identified according to the taxonomic interpretations of Gerdemann and Trappe (1974). The two spore types most commonly observed in the wet-sieved soil were identified as *Glomus fasciculatus* (Thaxt. sensu Gerd.) Gerdemann and Trappe, and *Glomus mossae* (Nicol. and Gerd.) Gerdemann and Trappe. Spores of *G. fasciculatus* were more abundant than spores of *G. mosseae*. Spores of both species were surface-sterilized in 0.05% sodium hypochlorite for 10 minutes and rinsed three times in

distilled water prior to being introduced as a mixture into pot cultures.

Initial pot cultures were established in surface-sterilized 14 cm plastic pots in the greenhouse. Approximately 75 to 100 surface-sterilized spores were pipetted in a layer within a sand-soil medium (2:1, v/v) that had been steam sterilized for two hours on each of two consecutive days. Seeds of sudangrass (*Sorghum vulgare* Pers.) were surface sterilized for 10 minutes in 0.5% sodium hypochlorite, rinsed three times in distilled water, and then placed on the surface of inoculated pot culture medium and covered with a 2 cm layer of sterilized sand-soil medium. Pot cultures were fertilized with 250 ml per pot of water-soluble fertilizer (172 ppm N, 75 ppm P, and 160 ppm K) once every month and watered sparingly by hand. Supplemental flourescent lighting was used to extend the day length to 16 hours. After four months, aboveground portions of the host plants were removed and discarded, and the pot culture medium was mixed and used to inoculate the plant growth medium for container-grown plants.

Inoculation of Experimental Plants

Approximately 250 cc of pot culture inoculum, containing propagules (spores, infected rootlets) of *G. fasciculatus* and *G. mosseae*, were placed in two layers within container medium (peat moss–perlite–sand–soil, 1:1:2:4, v/v; steam sterilized for two hours on each of two consecutive days) in 5 × 5 × 25 cm, paraffin-coated, paperboard containers. Surface-sterilized seeds (same treatment as used for sudangrass seeds) of *A. canescens* were placed in inoculated and noninoculated containers, and after development of the first set of true leaves, seedlings were thinned to one plant per container. Noninoculated seedlings were initially watered with a leachate from the pot culture inoculum to introduce soil microorganisms other than VAM fungi. Inoculated and noninoculated *A. canescens* seedlings were then fertilized in the same manner as *S. vulgare* plants and watered sparingly by hand. Supplemental flourescent lighting was used to extend day-length to 16 hours. Plants were

hardened-off over a two-week period outside the greenhouse.

Inoculated and noninoculated plants were assessed for mycorrhizal development prior to transplanting and at the termination of the experiment. Root samples were cleaned of debris, cut into 1-cm segments, cleaned in 10% KOH, and stained in .05% trypan-blue in lacto-phenol (Phillips and Hayman 1970). Stained root segments were mounted in clear lacto-phenol and examined under a compound microscope at 140X for the presence or absence of VAM infection. A root segment was considered infected if it contained arbuscules, vesicles, or peletons, or any combination of the three. Percentage infection for a plant was calculated as the number of segments with any infection out of a random sample of 100 segments.

Experimental Procedures

Inoculated and noninoculated plants, ranging in height from 15 to 17 cm, were outplanted 5 June 1979 on processed shale and disturbed soil at the Anvil Points site. The split-plot design had five plants per treatment. Screen-caged, single junction, thermocouple psycrometers (J. D. R. Merrill Specialty Equipment Co., Logan, Utah) were placed beneath each plant at a depth of 30 cm (approximately 5 cm below each soil plug) to measure soil/shale water potentials. Plants in every treatment were fertilized with 34 kg/ha N as NH_4NO_3 (granular 34-0-0) and 34 kg/ha P as $Ca(H_2PO_4)_2 \cdot H_2O$ (granular 0-46-0) and given 1 l of water. Plant height and soil/shale water potentials were recorded at approximately three-week intervals during the two growing seasons. A Wescor HR-33T Dew Point Microvoltmeter (Wescor, Inc., Logan, Utah) was used to measure the microvolt output of each psychrometer after a 15-second cooling period. All readings were taken at dawn.

Plant water potentials were also measured at three-week intervals during the second growing season. Salt accumulation on the external leaf surface of *A. canescens* made it impossible to accurately measure plant water potential by psychrometric techniques. Therefore, plant water potential was measured with a pressure bomb (Soil Moisture

Equip. Corp., Santa Barbara, California) using the method of Waring and Cleary (1967). Cut stem tips (about 10 cm long), from the same approximate location on each plant, were placed in a pressure bomb and pressure was applied until sap first appeared at the cut end. All readings were taken at dawn.

Survival was measured in September of the first growing season (1979) and in June and September of the second growing season (1980). Plant cover was measured with a .25 m² quadrat prior to excavating plants from the soil and processed shale. Following excavation, aboveground biomass was dried at 80 C for 48 hours, weighed, ground in a Thomas-Wiley mill (1 mm mesh screen), and digested according to the method of Chapman and Pratt 1961). Phosphorus content of digested biomass was determined by atomic absorption spectrophotometry (Chapman and Pratt 1961) and total N content was determined by a modified Kjeldahl method (Horowitz 1965).

Samples of Paraho processed oil shale and disturbed native soil from the Anvil Points field site were analyzed for texture, pH, electrical conductivity, phosphorus, nitrate-nitrogen, potassium, sodium, calcium, and magnesium by the Soil Analysis Laboratory at Utah State University (Table 1).

Statistical Interpretations

Analysis of variance was used to determine the statistical significance ($P < 0.05$) of data from the split-plot field experiment. The least significant difference (LSD) test was applied to field data to determine significant differences ($P < 0.05$) in aboveground biomass, plant height, plant cover, and percent P and percent N of shoot material, and significant differences in soil/shale water potentials and plant water potentials between in-

culation treatments and media treatments for each sampling date. Only the main effects (inoculation method and media type) were statistically significant at an acceptable level ($P < 0.05$). As a result, the analysis of differences among inoculation treatments were summed across media treatments and vice versa.

Results and Discussion

Plant Growth Responses

After two growing seasons in the field, aboveground biomass, height, and cover of plants inoculated with VAM fungi were respectively 2.5, 1.8, and 2.2 times greater than those of noninoculated controls (Table 2). Significant differences were also observed between plants growing in disturbed soil and processed shale. Aboveground biomass, height, and cover of plants growing in soil were respectively 2.2, 1.8, and 1.8 times greater than those of plants growing in processed shale (Table 2). The low production of aboveground biomass and the small increase in height of plants in processed shale can be primarily attributed to the low water-holding capability, high EC, high pH, and general nutrient deficiency of the spent shale.

The growth responses observed in this experiment were similar to those of Williams et al. (1974) and Aldon (1978), who investigated the effects of VAM on the growth of *A. canescens* in soil and coal mine spoil material in New Mexico. In field and greenhouse studies, plants inoculated with *Glomus mosseae* had greater aboveground biomass in soil (Williams et al. 1974) and greater height and cover in coal mine spoil material (Aldon 1978) than noninoculated controls.

There were no differences in survival rates between inoculated and noninoculated plants

TABLE 1. A comparison of physical and chemical properties of Paraho processed shale and native soil from the study site at Anvil Points, Colorado.

| | Texture | pH° | ECₑ°° mmhos/cm | meq/l | | | ppm | | |
				Na†	Ca + Mg†	p‡	NO₃π	K†
Paraho Colorado shale	Silty gravel	8.6	8.0	52.2	92.3	3.8	2.2	293
Disturbed native soil	Channery loam	7.9	6.7	67.4	26.5	3.1	8.7	80

°Based on saturated paste
°°Based on saturation extract
†Ammonium acetate extractable
‡Sodium bicarbonate extractable
π Phenodisulfonic acid extractable

in both types of media. All treatments had survival rates of 100 percent due in part to the favorable field conditions. In the 25-day period following field outplanting on 5 June 1979, *A. canescens* transplants received 11.7 mm of precipitation. This precipitation, in addition to the 1 *l* of supplemental water applied at the time of outplanting, may have overshadowed the effects of VAM in reducing transplant shock.

Nutritional Status

Plants inoculated with VAM fungi had significantly higher P contents and slightly higher N contents than noninoculated controls (Table 2). Plants growing in disturbed soil had slightly higher P contents and significantly higher N contents than plants growing in processed shale (Table 2).

Soils and spoils such as those derived from shales in northeastern Utah and northwestern Colorado are usually deficient in plant-available P (Bauer et al. 1978). Increased growth associated with mycorrhizal infection in nutrient-deficient soils and spoils has been attributed to enhanced nutrient uptake, especially the uptake of P (Aldon 1978, Daft and Nicolson 1966, Gerdemann 1975). Fungal hyphae extending from mycorrhizal roots compensate for deficiencies of immobile ions such as phosphate by exploring a greater volume of soil than roots alone, and by presenting a greater surface area for phosphate uptake (Gerdemann 1975). In addition to

TABLE 2. Biomass, height, cover, and P and N contents of aboveground plant material after two growing seasons in the field for inoculated and noninoculated *A. canescens* plants grown in processed shale and disturbed soil.[*]

	Inoculation[**]		Media[**]	
	+ M‡	–M‡	Soil	Shale
Shoot				
biomass (g)	60.89a	24.51b	58.89e	26.59f
Height (cm)†	33.50a	18.50b	33.35e	18.65f
Cover (cm²)	1192a	552b	1125e	620f
P (%)	0.095a	0.071b	0.089e	0.076e
N (%)	1.76a	1.71a	1.97e	1.51f

[*]Values are means of five replicates.
[**]Means in the same row followed by the same letter are not significantly different at the 0.05 level.
‡ + M = inoculation with mycorrhizal fungi, and –M = no inoculation with mycorrhizal fungi.
†Values represent increases in plant height above a base height of 15 to 17 cm at time of transplanting.

increasing the surface area of infected roots, VAM fungi take up phosphate much more rapidly than nonmycorrhizal roots and transfer it rapidly to the host plant via the relatively large surface area of the arbuscules (Bowen et al. 1975).

The forms and amounts of N that are available on a seasonal basis to plants in arid and semiarid environments have not been satisfactorily documented (Wallace et al. 1978). Some studies indicate that NO_3 is the major form of N taken up and assimilated by several salt desert shrub species under greenhouse conditions (Institute for Land Rehabilitation 1979) and field conditions (Wallace 1978). Nitrate moves to the root by mass flow under most conditions. Where the transfer of ions is rapid as in mass flow, uptake will be limited mainly by the absorbing capacity of the root and VAM (Bowen and Smith 1981). As a result, hyphal proliferation from mycorrhizal roots may have less effect on the uptake of NO_3 than on the uptake of P.

Soil/Shale and Plant Water Relations

During the first growing season (1979) of the field experiment, soil/shale water potentials were significantly lower under mycorrhizal transplants than under nonmycorrhizal transplants from 17 July through 9 September (Fig. 1A). The lower water potentials indicate that roots from mycorrhizal transplants may have grown more actively into soil and processed shale than roots from nonmycorrhizal transplants and extracted available soil/shale moisture near the psychrometers. It is possible that the roots (and hyphae) of inoculated plants could have reached the immediate vicinity of the psychrometers (approximately 5 cm away) within a month after outplanting since roots of a closely related species, *Atriplex confertifolia*, have been reported to grow at the rate of approximately 2 to 3 mm per day during June and July in a cool desert environment (Fernandez and Caldwell 1977). Similar trends in soil/shale water potentials were noted for inoculation treatments and media treatments during the second growing season (1980) (Figs. 1A and 1B).

Soil/shale water potential data also indicated that processed shale was a more limiting growth medium than native soil in
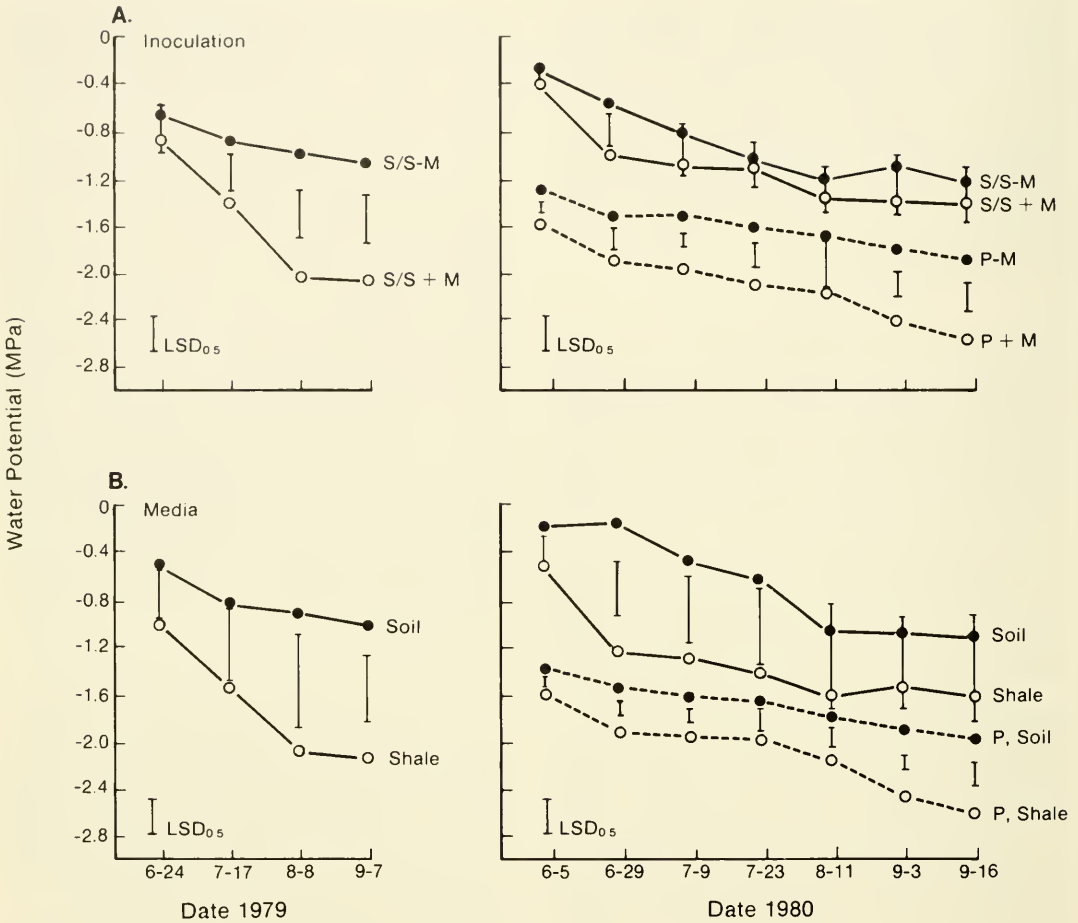
Fig. I. Soil/shale water potentials and plant water potentials during two field-growing seasons for *A. canescens*: (A) inoculated two ways (S/S–M = soil/shale water potential under noninoculated plants, S/S + M = soil/shale water potential under inoculated plants, P–M = water potential of noninoculated plants, and P + M = water potential of inoculated plants), and (B) grown in processed shale and disturbed soil (SOIL = soil water potential under inoculated and noninoculated plants, SHALE = shale water potential under inoculated and noninoculated plants, P,SOIL = water potential of inoculated and noninoculated plants in soil, and P,SHALE = water potential of inoculated and noninoculated plants in shale).

terms of water relations (Fig. 1B). The spent shale had a higher electrical conductivity than the native soil (Table 1), and thus the osmotic potential of the shale solution was more negative than that of the soil solution. The permeability of the spent shale layer was much lower than that for the disturbed channery loam in the trench. Salts, especially Na, accumulated at the surface (EC_e of 540 mmhos/cm; McKell, unpubl. data) via capillary rise and destroyed the physical structure of the spent shale by dispersion of the fine particles (Striffler et al. 1974). The per-

meability of the spent shale surface was further reduced when crusting occurred as the shale dried.

Plant water potentials followed the seasonal progression of soil/shale water potentials during the second growing season (Figs. 1A and 1B). Plant water potentials were significantly lower for mycorrhizal plants than nonmycorrhizal controls for all sampling dates except 11 August (Fig. 3A). Mycorrhizal plants had greater leaf surface area for transpirational loss, and thus developed steeper gradients in water potential (from

leaf to soil) than nonmycorrhizal plants. Differences in aboveground biomass may not completely account for higher transpiration rates in *A. canescens* plants inoculated with VAM fungi. In a study involving similarly sized, 8-month-old, mycorrhizal and non-mycorrhizal rough lemon (*Citrus jambhiri* Lush.) seedlings, Levy and Krikun (1980) found that mycorrhizal plants had slightly higher transpiration flux densities and stomatal conductance than nonmycorrhizal plants during a four-day period of imposed water stress and a four-day recovery period following rewatering. In that study (Levy and Krikun 1980), as in another (Allen et al. 1981) with blue grama (*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.), the influence of the mycorrhizal association on plant water relations was attributed, in part, to changes in phytohormone levels.

Several investigators have studied the plant-soil water relations of *Atriplex* species in the greenhouse and in their native cool desert environments. Richardson and McKell (1980) demonstrated that *A. canescens*, growing in processed oil shale in the greenhouse, was able to adjust osmotic potentials so as to maintain positive turgor at plant water potentials exceeding –4.0 MPa. Near Federal Oil Shale Lease Tracts Ua and Ub in northeastern Utah, Barker (1978) observed measurable shoot growth for one-year-old container-grown transplants of *A. canescens* growing in soil with a water potential of –2.2 MPa at a depth of 30 cm. Love and West (1972) measured plant water potentials of up to –8.0 MPa in *A. confertifolia* in northern Utah during July and August. During July and August in the same area, other investigators working with *A. confertifolia* observed measurable root growth at –8.0 MPa soil water potential at a depth of 40 cm (Fernandez and Caldwell 1977), and positive net photosynthesis at plant water potentials exceeding –9.0 MPa (White 1976). These studies indicate that *A. canescens* plants outplanted in this study were well within their limits, and thus the effect of VAM on drought tolerance may not have been expressed fully or at all. However, VAM may have permitted greater root extension and use of available water during the establishment of these transplants.

Mycorrhizal Development

The extent of mycorrhizal infection is of importance when studying the influence of VAM on the host plant. After two growing seasons in the field, the infection percentage of inoculated plants decreased from 13.0 at outplanting to 9.8 in disturbed soil and 3.8 in processed shale. Only 0.8 percent of the root segments examined from nonmycorrhizal controls growing in disturbed soil showed evidence of infection. Nonmycorrhizal fourwing saltbush plants in processed shale showed no signs of infection. Difficulty was encountered during the excavation of roots, particularly fine root elements, from the processed shale pile and the soil trench. Fine root elements constitute the major portion of mycorrhizal roots on native shrub species (Staffeldt and Vogt 1975) and, hence, infection percentages may have been underestimated.

Water-soluble, retorted oil shale constituents probably contributed to the decline in infection of inoculated plants growing in processed shale. Hersman and Klein (1979) demonstrated that the addition of 25% processed shale to topsoil caused significant decreases in fungal populations and soil microbial activities. In another study (DeVore and Christensen 1979), root microfungi numbers were significantly reduced after in situ oil shale process water was applied to *Artemisia tridentata* plots. However, a fairly diverse microflora survived four months after the application of the process water.

Even with low infection percentages, inoculated plants still displayed positive growth responses. High infection may not be a prerequisite for growth response in all plants inoculated with VAM fungi. Abundant VAM can be present with no subsequent detectable growth difference and, conversely, a low infection level can sometimes induce marked growth stimulation (Daft and Nicolson 1966).

We conclude that VAM help make processed oil shale a more tractable medium for the establishment of plants representative of later successional stages by allowing these plants to make effective use of the natural resources that are limiting under conditions of

high stress. The success of reestablishing a diverse, functional plant community that is capable of long-term stability without continued inputs of water and fertilizer may depend, in part, on success in the reinoculation and manipulation of essential microorganisms (including VAM fungi) in the initially sterile shale.

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